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siRNA transfection in Calu-3 cells

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An abbreviated version of this protocol was published in Mol Cell in Apr 2021

Functional landscape of SARS-CoV-2 cellular restriction

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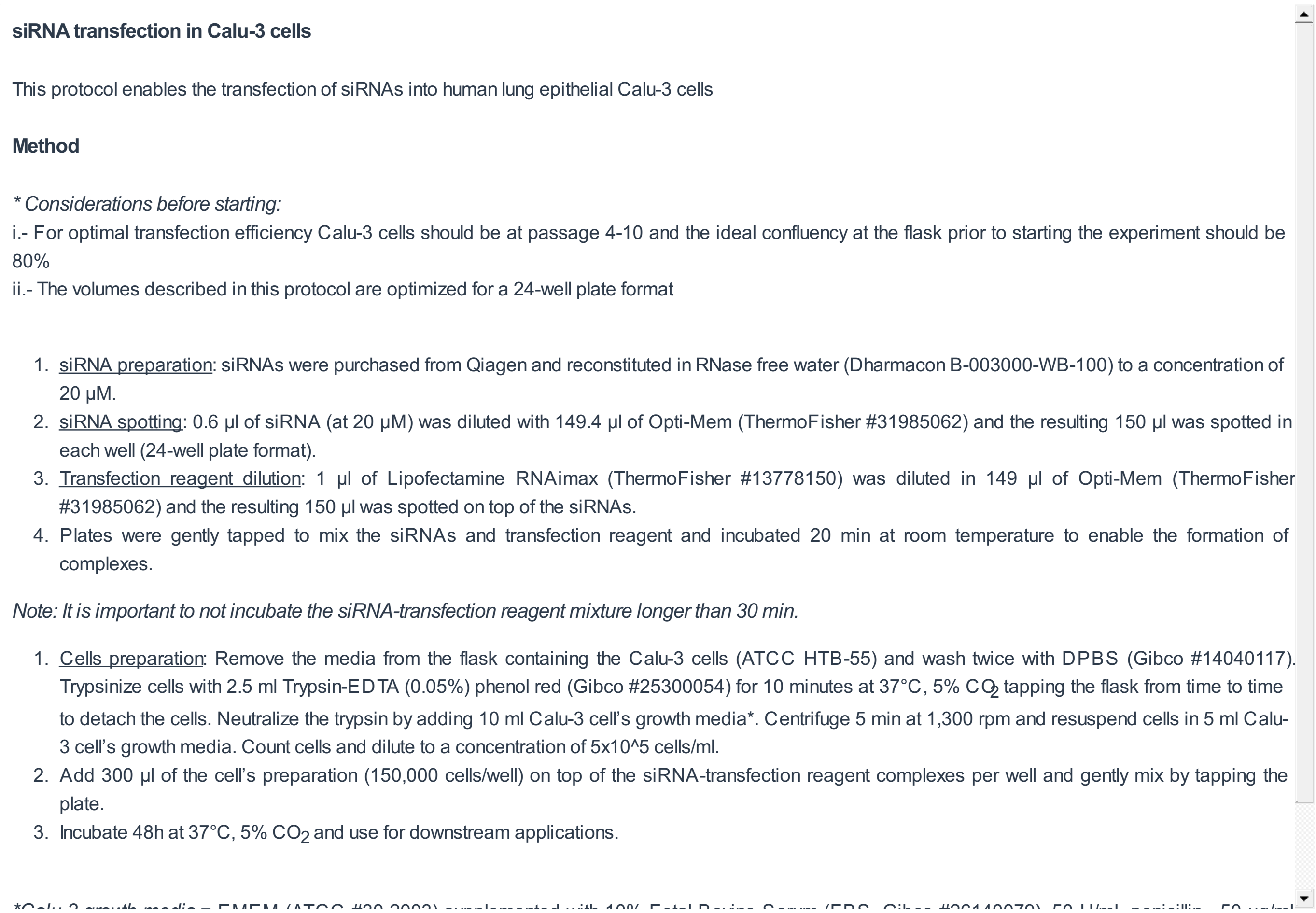
Detailed protocol



siRNA transfection in Calu-3 cells

This protocol enables the transfection of siRNAs into human lung epithelial Calu-3 cells

Method

** Considerations before starting:*
i.- For optimal transfection efficiency Calu-3 cells should be at passage 4-10 and the ideal confluency at the flask prior to starting the experiment should be 80%
ii.- The volumes described in this protocol are optimized for a 24-well plate format

1. siRNA preparation: siRNAs were purchased from Qiagen and reconstituted in RNase free water (Dharmacon B-003000-WB-100) to a concentration of 20 µM.
2. siRNA spotting: 0.6 µl of siRNA (at 20 µM) was diluted with 149.4 µl of Opti-Mem (ThermoFisher #31985062) and the resulting 150 µl was spotted in each well (24-well plate format).
3. Transfection reagent dilution: 1 µl of Lipofectamine RNAimax (ThermoFisher #13778150) was diluted in 149 µl of Opti-Mem (ThermoFisher #31985062) and the resulting 150 µl was spotted on top of the siRNAs.
4. Plates were gently tapped to mix the siRNAs and transfection reagent and incubated 20 min at room temperature to enable the formation of complexes.

Note: It is important to not incubate the siRNA-transfection reagent mixture longer than 30 min.

1. Cells preparation: Remove the media from the flask containing the Calu-3 cells (ATCC HTB-55) and wash twice with DPBS (Gibco #14040117). Trypsinize cells with 2.5 ml Trypsin-EDTA (0.05%) phenol red (Gibco #25300054) for 10 minutes at 37°C, 5% CO_2 tapping the flask from time to time to detach the cells. Neutralize the trypsin by adding 10 ml Calu-3 cell's growth media*. Centrifuge 5 min at 1,300 rpm and resuspend cells in 5 ml Calu-3 cell's growth media. Count cells and dilute to a concentration of 5x10^5 cells/ml.
2. Add 300 µl of the cell's preparation (150,000 cells/well) on top of the siRNA-transfection reagent complexes per well and gently mix by tapping the plate.
3. Incubate 48h at 37°C, 5% CO_2 and use for downstream applications.

How to cite:（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Martin-Sancho, L. and Chanda, S. (2023). siRNA transfection in Calu-3 cells. Bio-protocol Preprint. bio-protocol.org/prep2103.

2. Martin-Sancho, L., Lewinski, M. K., Pache, L., Stoneham, C. A., Yin, X., Becker, M. E., Pratt, D., Churas, C., Rosenthal, S. B., Liu, S., Weston, S., Jesus, P. D. D., O'Neill, A. M., Gounder, A. P., Nguyen, C., Pu, Y., Curry, H. M., Oom, A. L., Miorin, L., Rodriguez-Frandsen, A., Zheng, F., Wu, C., Xiong, Y., Urbanowski, M., Shaw, M. L., Chang, M. W., Benner, C., Hope, T. J., Frieman, M. B., García-Sastre, A., Ideker, T., Hultquist, J. F., Guatelli, J. and Chanda, S. K.(2021). Functional landscape of SARS-CoV-2 cellular restriction. Mol Cell 0(0). DOI: 10.1016/j.molcel.2021.04.008

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